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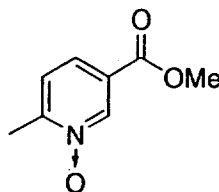
## Efficient DNA Cleavage with an Iron Complex Without Added Reductant

Gerard Roelfes, Mark E. Branum, Li Wang, Lawrence Que, Jr. \* and Ben L. Feringa\*

### Supporting Information

#### General information

Di(2-pyridinyl)methylamine<sup>1</sup> and 9-chloroacridine<sup>2</sup> were prepared according to literature procedures. The Litmus 29 plasmid DNA (New England Biolabs), prepared from DH5 $\alpha$  cells, was purified using the Qiagen Plasmid Maxi Kit.

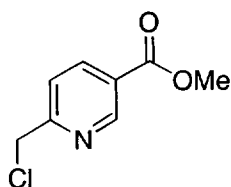


#### Methyl 6-methylnicotinate *N*-oxide

Methyl 6-methylnicotinate (10 g, 66.2 mmol) was dissolved in dichloromethane (150 ml). 3-Chloroperoxybenzoic acid (17 g, 112 mmol) was added and the mixture was stirred for 3 h at room temperature. Saturated NaHCO<sub>3</sub> solution (200 ml) was added and the mixture was stirred for an additional hour. The dichloromethane layer was separated and the aqueous layer was extracted with dichloromethane (2  $\times$  100 ml). The combined dichloromethane layers were washed with saturated NaHCO<sub>3</sub> (aq) (100 ml), brine (100 ml) and dried (Na<sub>2</sub>SO<sub>4</sub>). After evaporation of the solvent methyl 6-methylnicotinate *N*-oxide (7.8 g, 51.0 mmol, 77 %) was obtained as a creme colored solid, mp 90.4 – 90.8 °C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  2.52 (s, 3H), 3.90 (s, 3H), 7.32 (d, 1H, *J* = 8.05 Hz), 7.70 (dd, 1H, *J* = 8.05 Hz, *J* = 1.1 Hz), 8.80 (d, 1H, *J* = 1.1 Hz); HRMS calcd. for C<sub>8</sub>H<sub>9</sub>NO<sub>3</sub> 167.058, found 167.060.

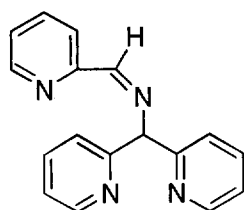
Buffers used: Mes (pH 5.7), Bistris (pH 6.5), Hepes (pH 7.2), Tris (pH 8.0),

Ches (pH 9.2); reactions carried out at 37 °C, using 10 mM buffer concentration.



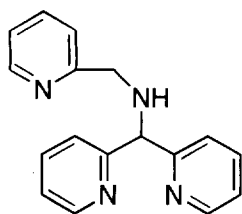
### Methyl 6-(chloromethyl)nicotinate

*p*-Toluenesulfonyl chloride (10.7 g, 56.1 mmol) was combined with methyl 6-methylnicotinate *N*-oxide (7.8 g, 51.0 mmol) in dioxane (100 ml) under an Argon atmosphere. The reaction mixture was heated under reflux for 1 night. After cooling to room temperature the solvent was evaporated and the residue dissolved in dichloromethane (200 ml). The solution was washed with saturated Na<sub>2</sub>CO<sub>3</sub> (aq) (2 × 100 ml), brine (50 ml) and dried (Na<sub>2</sub>SO<sub>4</sub>). After evaporation of the solvent the product was purified by column chromatography (SiO<sub>2</sub>, hexane/ethyl acetate 10:2.5, *R*<sub>f</sub> = 0.4) to give methyl 6-(chloromethyl)nicotinate (5.71 g, 30.8 mmol, 60 %) as a slightly yellow solid. An analytically pure sample could be obtained by recrystallization from *n*-hexane, mp 63.5 – 63.8 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 3.94 (s, 3H), 4.70 (s, 2H), 7.58 (d, 1H, *J* = 8.4 Hz), 8.30 (dd, 1H, *J* = 8.1 Hz, *J* = 2.2 Hz), 9.08 (d, 1H, *J* = 1.5 Hz); Anal. Calcd. for C<sub>8</sub>H<sub>8</sub>ClNO<sub>2</sub>: C 51.77, H 4.34, N 7.55; found: C 51.50, H 4.23, N 7.46.



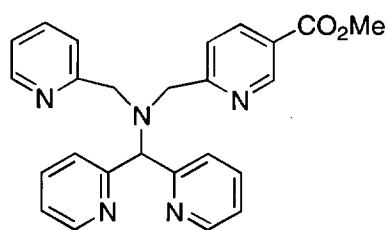
### *N*-[di(2-pyridinyl)methylidene]-*N*-(2-pyridinylmethyl)amine

To di-2-pyridyl methyl amine (1.5 g, 8.1 mmol) was added freshly distilled pyridine-2-carboxaldehyde (900 mg, 8.4 mmol). After shaking the flask, the mixture was allowed to stand for approximately 2 hours. The white solid was collected and washed with cyclohexane to remove traces of unreacted starting material to give pure *N*-[di(2-pyridinyl)methylidene]-*N*-(2-pyridinylmethyl)amine (2.02 g, 91 %). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 6.0 (s, 1H), 7.16 (m, 2H), 7.31 (m, 1H), 7.59 (m, 5H), 8.18 (m, 1H), 8.61 (m, 3H), 8.65 (s, 1H); MS (CI): *m/z* 275 (*M*+1).



### ***N*-[di(2-pyridinyl)methyl]-*N*-(2-pyridinylmethyl)amine**

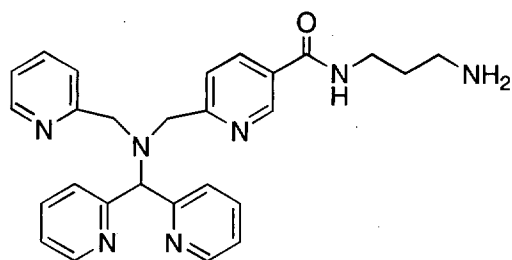
To a solution of *N*-[di(2-pyridinyl)methylidene]-*N*-(2-pyridinylmethyl)amine (1.5 g, 5.5 mmol) in methanol (20 ml) was added NaBH<sub>4</sub> (0.45 g, 11.8 mmol) in small portions. After stirring at room temperature for 2 hours HCl (aq) is added until the pH<2. After stirring for 30 min 5 N NaOH (aq) was added until the pH>9. The methanol was removed through evaporation and the aqueous layer was extracted with ethyl acetate (3 ×30 ml). The combined ethyl acetate layers were washed with brine (30 ml) and dried (Na<sub>2</sub>SO<sub>4</sub>). Evaporation of the solvent gave *N*-[di(2-pyridinyl)methyl]-*N*-(2-pyridinylmethyl)amine (1.35 g, 89 %) as a yellow oil. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 3.85 (s, 2H), 5.10 (s, 1 H), 7.03 (m, 3H), 7.41 (m, 6H), 8.46 (m, 3H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 53.1 (t), 68.9 (d), 121.8 (d), 122.1 (d), 122.2 (d), 122.3 (d), 136.3 (d), 136.5 (d), 149.2 (d), 159.6 (s), 161.2 (s); MS (CI): m/z 277 (M+1).



### **6-(((di-pyridin-2-yl-methyl)-pyridin-2-ylmethyl-amino)-methyl) nicotinic acid methyl ester**

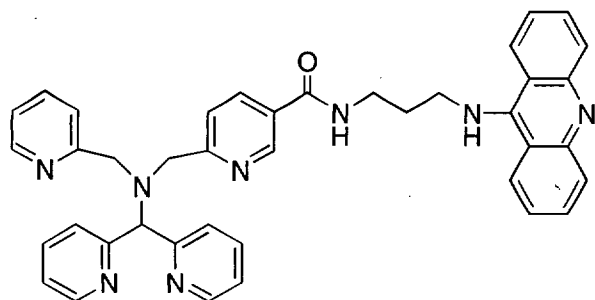
A solution of *N*-[di(2-pyridinyl)methyl]-*N*-(2-pyridinylmethyl)amine (1.45 g, 5.3 mmol), methyl 6-(chloromethyl)nicotinate (1.08 g, 5.8 mmol) and *N,N*-diisopropylethylamine (1.3 ml, 7.5 mmol) in acetonitrile (20 ml) was heated under reflux overnight, under an Argon atmosphere. After cooling to room temperature the solvent was evaporated and the residue was purified by column chromatography (Al<sub>2</sub>O<sub>3</sub> neutral akt. I, ethyl acetate/hexane/triethylamine 10:5:1, R<sub>f</sub> = 0.5) to give 6-(((di-pyridin-2-yl-methyl)-pyridin-2-ylmethyl-amino)-methyl) nicotinic acid methyl ester (1.96 g, 84 %) as a dark oil. <sup>1</sup>H-NMR

(CDCl<sub>3</sub>)  $\delta$  3.91 (s, 3H), 3.95 (s, 2H), 4.05 (s, 2H), 5.33 (s, 1H), 7.11 (m, 3H), 7.65 (m, 7H) 8.20 (m, 1H), 8.48 (d, 1H,  $J = 4.9$  Hz), 8.56 (d, 2H,  $J = 4.9$  Hz), 9.06 (d, 1H,  $J = 2.2$  Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  52.17 (q), 57.18 (t), 57.56 (t), 72.31 (d), 121.91 (d), 122.19 (d), 122.21 (d), 122.39 (d), 123.04 (d), 123.93 (d), 124.06 (s), 136.33 (d), 137.32 (d), 149.12 (d), 149.34 (d), 150.20 (d), 159.42(s), 159.82 (s), 164.97 (s), 166.13 (s) ; MS (CI):  $m/z$  426 (M+1).



***N*-(3-amino-propyl)-6-(((di-pyridin-2-yl-methyl)-pyridin-2-ylmethyl-amino)-methyl) nicotinamide (L2)**

A solution of 6-(((di-pyridin-2-yl-methyl)-pyridin-2-ylmethyl-amino)-methyl) nicotinic acid methyl ester (473 mg, 1.11 mmol), 1,3-diaminopropane (1.1 ml, 13.1 mmol) and NaCN (7 mg, 0.14 mmol) in methanol (15 ml) was heated under reflux for 24 hours under an Argon atmosphere. After cooling to room temperature the mixture was poured into water (100 ml) and the aqueous layer was washed with ether (2  $\times$  125 ml), followed by extraction with dichloromethane (3  $\times$  75 ml). The combined dichloromethane layers were washed with water (50 ml), brine (50 ml) and dried (Na<sub>2</sub>SO<sub>4</sub>). Evaporation of the solvent afforded **L2** (418 mg, 81%) as a slightly yellow sticky solid. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  1.71 (m, 2H), 2.91 (m, 2H), 3.54 (m, 2H), 3.91 (s, 2H), 3.96 (s, 2H), 5.29 (s, 1H), 7.09 (m, 3H), 7.60 (m, 7H), 8.03 (m, 1H), 8.43 (d, 1H,  $J = 4.4$  Hz), 8.52 (d, 2H, 4.8 Hz), 8.85 (s, 1H); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  30.41 (t), 39.34 (t), 40.51 (t), 56.79 (t), 57.14 (t), 71.86 (d), 121.81(d), 122.07 (d), 122.35 (d), 122.86 (d), 123.82 (d), 128.36 (s), 135.39 (d), 136.23 (d), 136.29 (d), 147.42 (d), 148.95 (d), 149.18 (d), 159.27 (s), 159.58 (s), 162.58 (s), 165.33 (s); MS (CI):  $m/z$  468 (M+1).



***N*-(3-(acridin-9-ylamino)-propyl)-6-(((di-pyridin-2-ylmethyl)-pyridin-2-ylmethyl-amino)-methyl) nicotinamide (**L1**)**

A mixture of **L2** (301 mg, 0.64 mmol), 9-chloroacridine (140 mg, 0.64 mmol) and phenol (1.5 g) was heated at 90 °C during 3 hours under an Argon atmosphere. After cooling to room temperature diethyl ether was added (50 ml) and the suspension was stirred for 30 min. The diethyl ether was decanted, fresh diethyl ether was added (20 ml) and the suspension was stirred for 15 min after which the diethyl ether was decanted. 1 N NaOH (50 ml) was added and the aqueous layer was extracted with dichloromethane (3 × 75 ml). The combined dichloromethane layers were washed with brine (50 ml), dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent removed under reduced pressure. The solid residue was dissolved in ethanol (15 ml) and dry HCl gas was bubbled through the solution for 30 min. Diethyl ether (10 ml) was added and the hygroscopic precipitate filtered under argon. 1 N NaOH (50 ml) was added to the salt and the aqueous layer was extracted with dichloromethane (3 × 50 ml). The combined dichloromethane layers were washed with brine (50 ml), dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent removed *in vacuo* to give **L1** (410 mg, 0.63 mmol, 99 %) as a yellow solid, mp >250 °C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 2.00 (m, 2H), 3.67 (m, 2H), 3.90 (m, 2H), 3.93 (s, 2H), 4.00 (s, 2H), 5.31 (s, 1H), 7.19 (m, 5H), 7.31 (m, 2H), 7.61 (m, 9H), 7.99 (m, 1H), 8.04 (m, 2H), 8.22 (d, 2H, J = 8.2 Hz), 8.47 (d, 1H, J = 4.4 Hz), 8.54 (d, 2H, J = 4.8 Hz), 8.85 (s, 1H); ES/MS: m/z 645 (M+1).

**[(**L1-H**)Fe(CH<sub>3</sub>CN)](ClO<sub>4</sub>)<sub>3</sub>·2H<sub>2</sub>O (**2**)**

A solution of 70 % perchloric acid (26 mg, 0.18 mmol) in methanol (2 ml) was added to **L1** (116 mg, 0.18 mmol). To this solution was added Fe(ClO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O (72 mg, 0.2 mmol) in acetonitrile (2 ml). The dark red solution was divided in three equal portions and placed in an ethyl acetate bath. After 1 night **2** was obtained as a red solid which was recrystallized by slow vapor diffusion of ethyl acetate into an acetonitrile solution of **2**. After 3-4 days **2** (115

mg, 61 %) was obtained as red crystals.  $^1\text{H-NMR}$  ( $\text{CD}_3\text{CN}$ )  $\delta$  2.33 (m, 2H), 3.69 (m, 2H), 4.23 (m, 2H), 4.44 (m, 4H), 6.34 (s, 1H), 7.10 (d, 1H,  $J = 8.1$  Hz), 7.14 (d, 1H,  $J = 8.1$  Hz), 7.36 (m, 2H), 7.49 (t, 1H,  $J = 7.3$  Hz), 7.70 (dt, 1H,  $J = 7.7$  Hz,  $J = 1.1$  Hz), 7.78 (d, 1H,  $J = 8.4$  Hz), 7.89 (m, 7H), 8.45 (d, 2H,  $J = 8.8$  Hz), 8.90 (d, 1H,  $J = 5.1$  Hz), 9.01 (d, 1H,  $J = 5.5$  Hz), 9.05 (br, 1H), 9.18 (d, 1H,  $J = 5.5$  Hz), 9.24 (d, 1H,  $J = 1.1$  Hz), 11.19 (br, 1H); ES/MS:  $m/z$  899  $[\text{M} - (\text{ClO}_4)^- - (\text{CH}_3\text{CN})]^+$ , 400  $[\text{M} - 2(\text{ClO}_4)^- - (\text{CH}_3\text{CN})]^{2+}$ ; Anal. Calcd for  $\text{C}_{42}\text{H}_{44}\text{Cl}_3\text{Fe}_1\text{N}_9\text{O}_{15}$ : C 46.84, H 4.12, N 11.70; found: C 46.99, H 4.17, N 11.70.

### **$[(\text{L2-H})\text{Fe}(\text{CH}_3\text{CN})](\text{ClO}_4)_3$ (3)**

To a solution of **L2** (97 mg, 0.21 mmol) in  $\text{CH}_3\text{CN}$  (4 ml) was added 70 % perchloric acid (32 mg, 0.22 mmol), followed by  $\text{Fe}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$  (82 mg, 0.22 mmol). The solution was placed in an ethyl acetate bath and after 3 days **3** (146 mg, 0.17 mmol, 81 %) was isolated as red crystals.  $^1\text{H-NMR}$  ( $\text{CD}_3\text{CN}$ )  $\delta$  1.98 (m, 2H), 3.07 (m, 2H), 3.52 (m, 2H), 4.36 (m, 4H), 6.33 (s, 1H), 7.06 (d, 1H,  $J = 7.7$  Hz), 7.14 (d, 1H,  $J = 8.4$  Hz), 7.32 (m, 3H), 7.67 (dt, 1H,  $J = 7.7$  Hz,  $J = 1.1$  Hz), 7.97 (m, 6H), 8.89 (d, 1H,  $J = 5.1$  Hz), 9.01 (d, 1H,  $J = 5.5$  Hz), 9.17 (d, 1H,  $J = 5.1$  Hz), 9.21 (d, 1H,  $J = 1.1$  Hz); ES/MS:  $m/z$  722  $[\text{M} - (\text{ClO}_4)^- - (\text{CH}_3\text{CN})]^+$ , 311.5  $[\text{M} - 2(\text{ClO}_4)^- - (\text{CH}_3\text{CN})]^{2+}$ ; Anal. Calcd for  $\text{C}_{29}\text{H}_{33}\text{Cl}_3\text{Fe}_1\text{N}_8\text{O}_{13}$ : C 40.37, H 3.87, N 13.00; found: C 40.26, H 3.86, N 13.05.

### **DNA cleavage experiments, typical procedure**

A solution of metal complex was added to a buffered (Tris, 10 mM, pH 8.0) solution of Litmus29 plasmid DNA (0.1  $\mu\text{g}/\mu\text{l}$ ) at 37 °C. When reducing agents were used they were always added last. At indicated times a sample (10  $\mu\text{l}$ ) was taken from the reaction and quenched with a solution of number III dye<sup>3</sup> (2  $\mu\text{l}$ , consisting of 0.04 % bromophenol blue, 0.04 % xylene cyanol FF, and 5 % glycerol) and immediately frozen in liquid nitrogen. All gels were run on 1.2 % agarose slab gels for 120 min at 70 V. Gels were stained with ethidium bromide and pictures taken with Polaroid 3000 type 667 film. Bands on the film were quantified using the software program scion image 3b.

### **End group analysis**

The 5'-end-labeled restriction fragments were prepared by treatment of Litmus29 plasmid DNA with 50 U of *Hind*III followed by ethanol precipitation. The DNA was treated with 3 U of calf intestinal phosphatase followed by heat treatment at 75 °C for 10 min in the presence

of 10 mM pH 8 EDTA. After phenol-chloroform-isoamyl alcohol treatment and ethanol precipitation the DNA was 5'-end-labeled with 2 U T4 polynucleotide kinase in the presence of [ $\gamma$ - $^{32}$ P]-ATP. The DNA was treated with phenol-chloroform-isoamyl alcohol and run through a Sephadex G-50 column equilibrated to pH 8 with Tris buffer. After NH<sub>4</sub>OAc/ethanol precipitation, the DNA was treated with 4 U of *Pvu*II and purified with a 6 % nondenaturing PAGE gel. DNA was visualized by exposure to x-ray film and purified from the gel using the crush and soak method.<sup>3</sup>

The 5'-end-labeled restriction fragments (800,000-1,000,000 cpm) were treated with the cleavage agent at the indicated concentration in the presence of 0.25  $\mu$ g/10  $\mu$ l unlabeled linear fragments of Litmus 29 in Tris buffer (10 mM, pH 8) in a total volume of 100  $\mu$ l. Where necessary DTT was added to give a final concentration of 1mM. After the incubation for the indicated times the samples were ethanol precipitated and suspended in 20  $\mu$ l H<sub>2</sub>O.

Maxam and Gilbert reactions followed standard microbiology protocols.<sup>3</sup> DNase reactions were carried out in Tris (10 mM) for 15 min with serial dilutions of DNase I. The samples were run on a 20 % denaturing PAGE 7 M urea gel for 210 min at 50 V, then exposed to Fuji RX x-ray film.

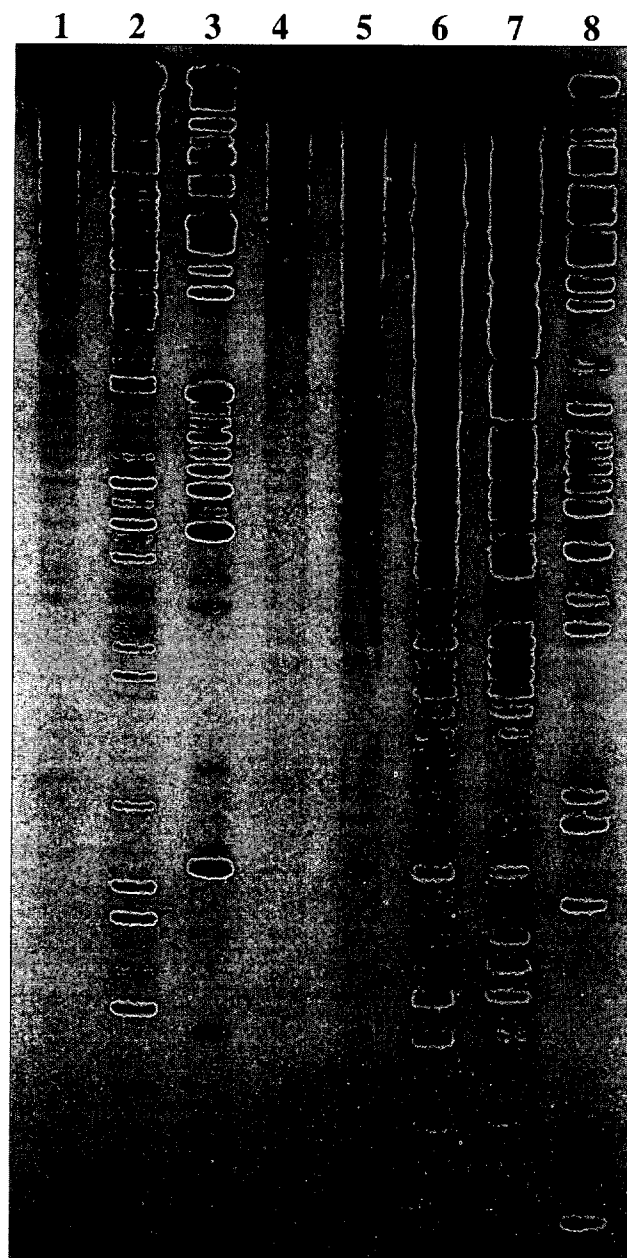
## References and Notes

<sup>1</sup> Niemers, E.; Hiltmann, R. *Synthesis* **1976**, 593-595.

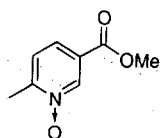
<sup>2</sup> Albert, A.; Ritchie, B. *Org. Synth., Coll. Vol. III*, **1955**, 53-56.

<sup>3</sup> Sambrook, J.; Fritsch, E. F.; Maniatus, T. *Molecular Cloning A Laboratory Manual*, 2nd Ed. Cold Spring Laboratory Press: Plainview, New York, **1989**.

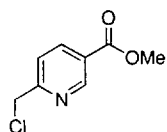
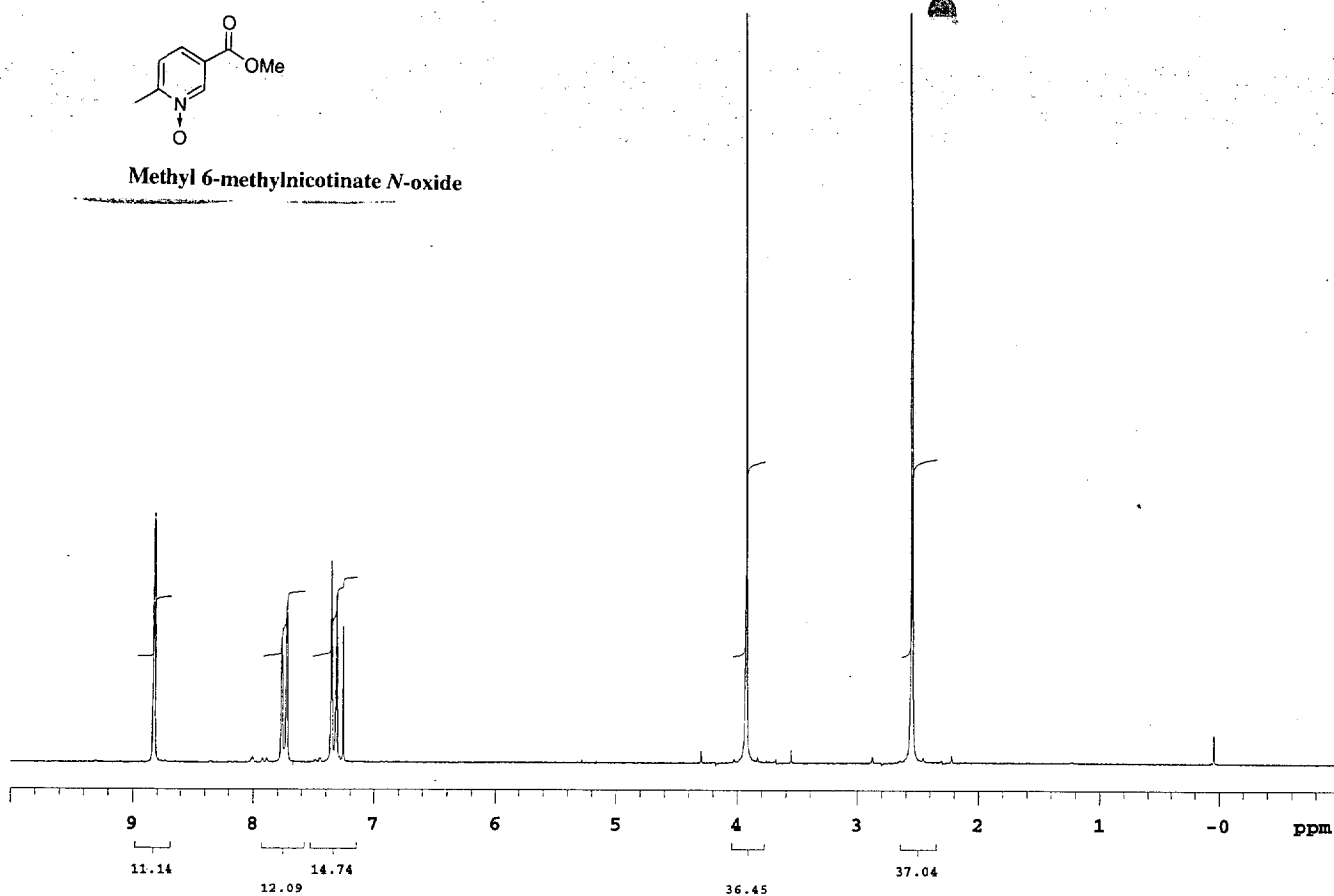




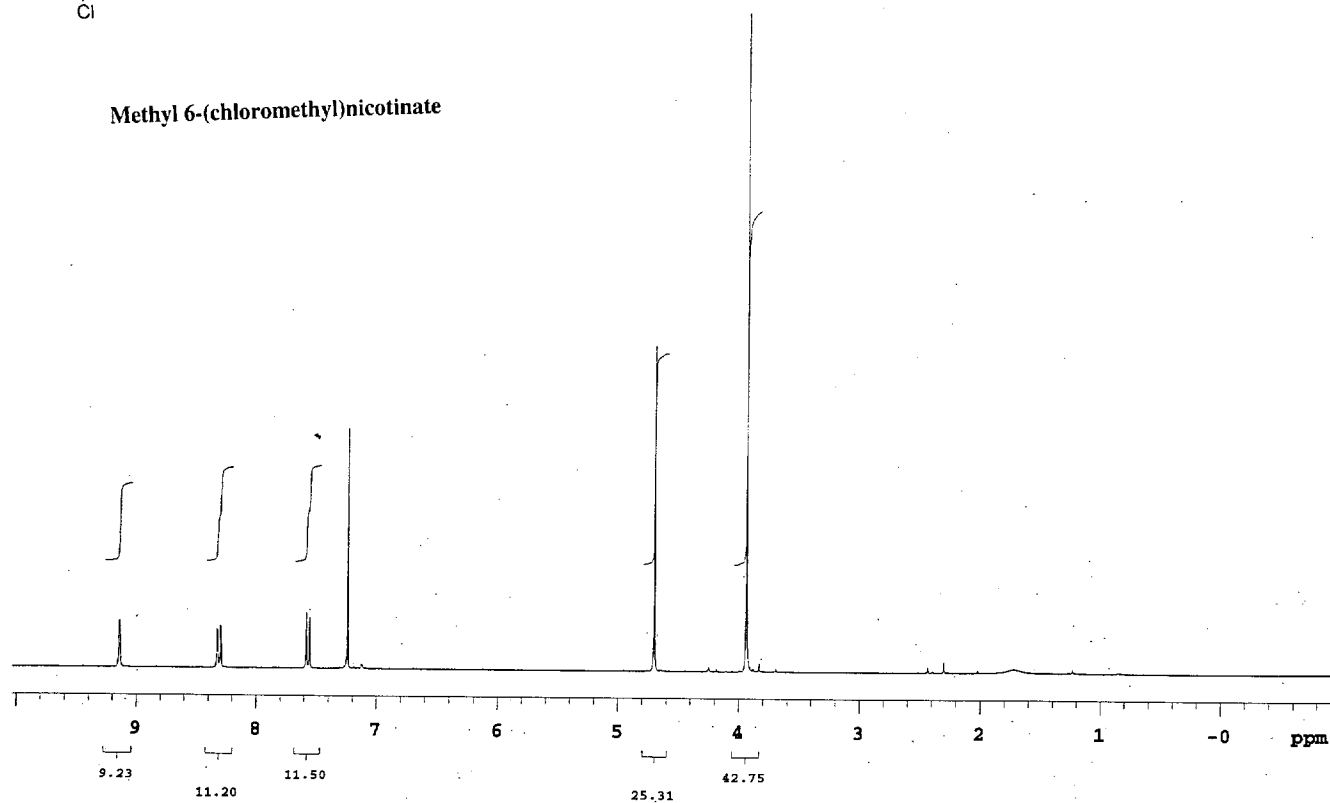
**Figure S1.** High-resolution denaturing PAGE gels of cleavage products of a 5'-<sup>32</sup>P-end-labeled 172-bp restriction fragment after 1h reaction. Cleavage reactions performed in Tris buffer (10 mM, pH 8.0) at 37 °C, without reducing agent. Lane 1, control; lane 2, DNaseI; lane 3, Fe-BLM (10 μM); lane 4, Fe-MPE (10 μM); lane 5, Fe-MPE (100 μM); lane 6, complex **2** (10 μM); lane 7, C & T; lane 8, G.

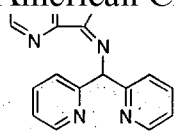


Methyl 6-methylnicotinate *N*-oxide

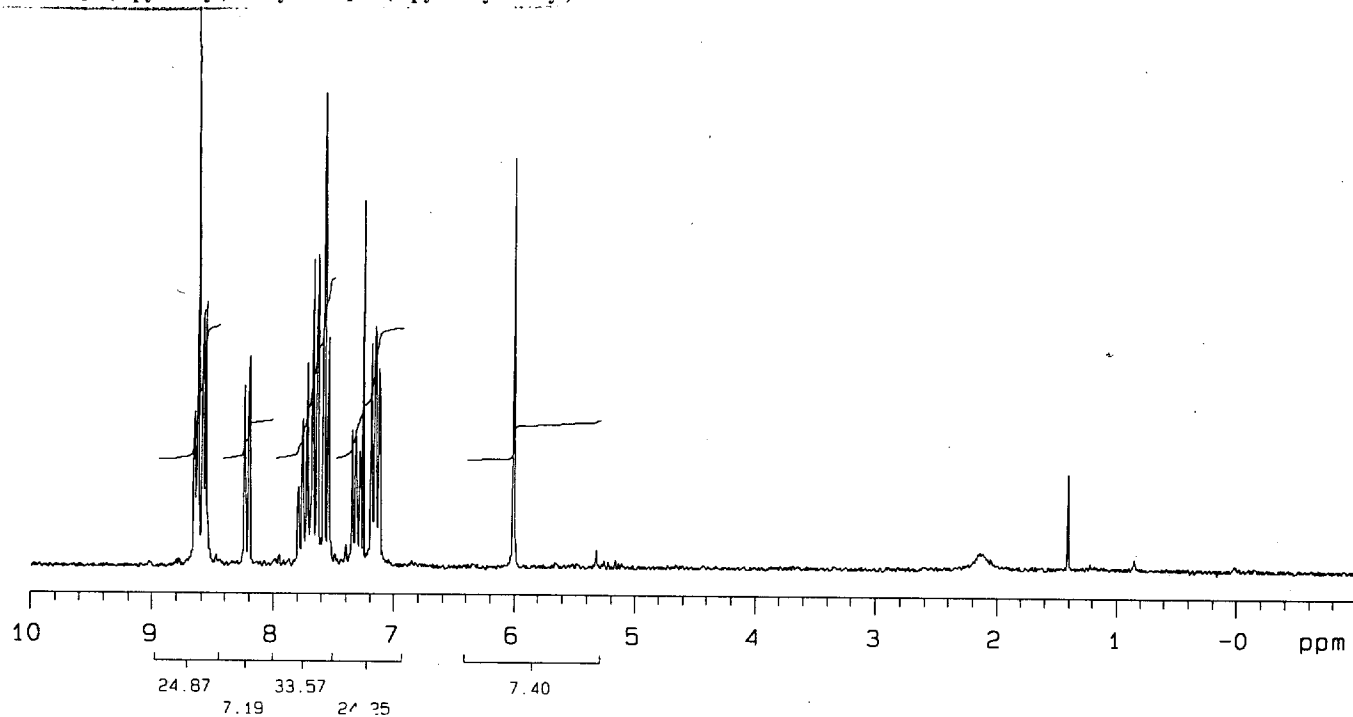


Methyl 6-(chloromethyl)nicotinate

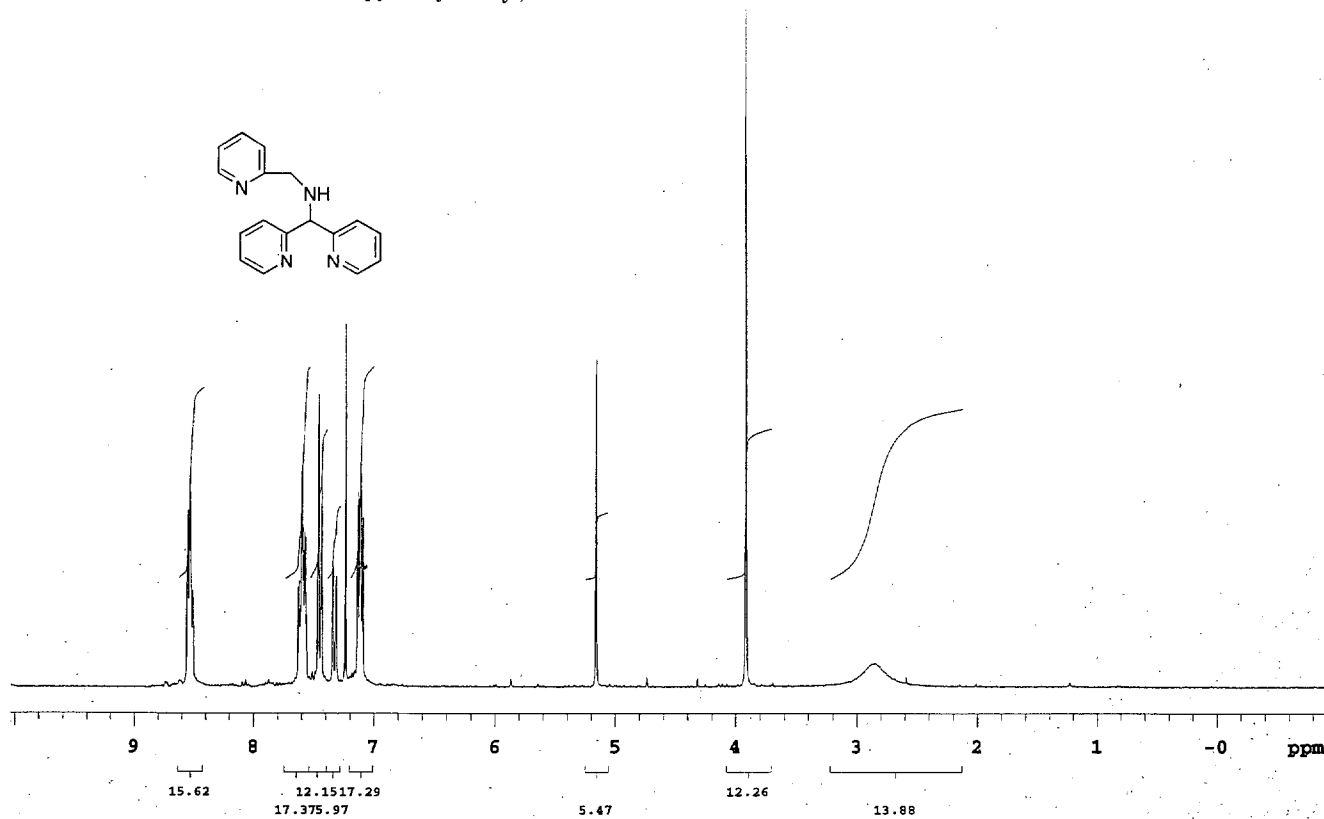
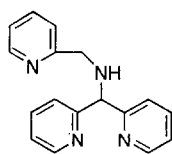


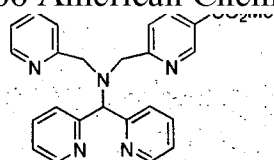


*N*-[di(2-pyridinyl)methylidene]-*N*-(2-pyridinylmethyl)amine

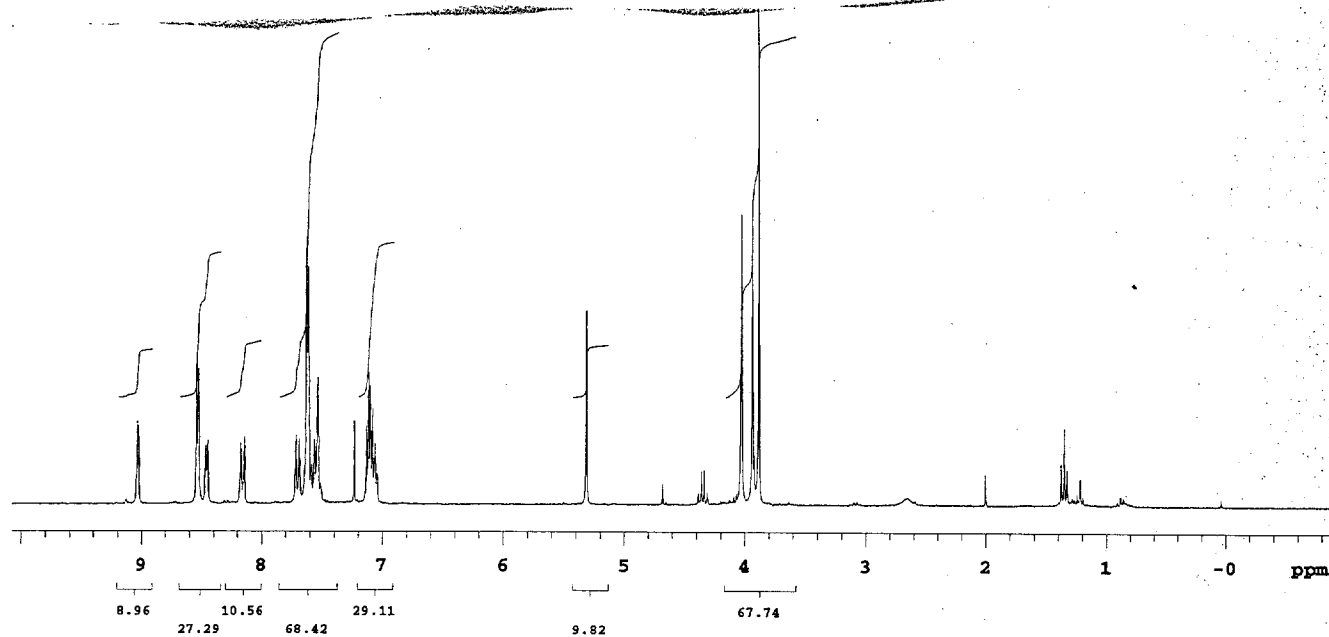


*N*-(di(2-pyridinyl)methyl)-*N*-(2-pyridinylmethyl)amine

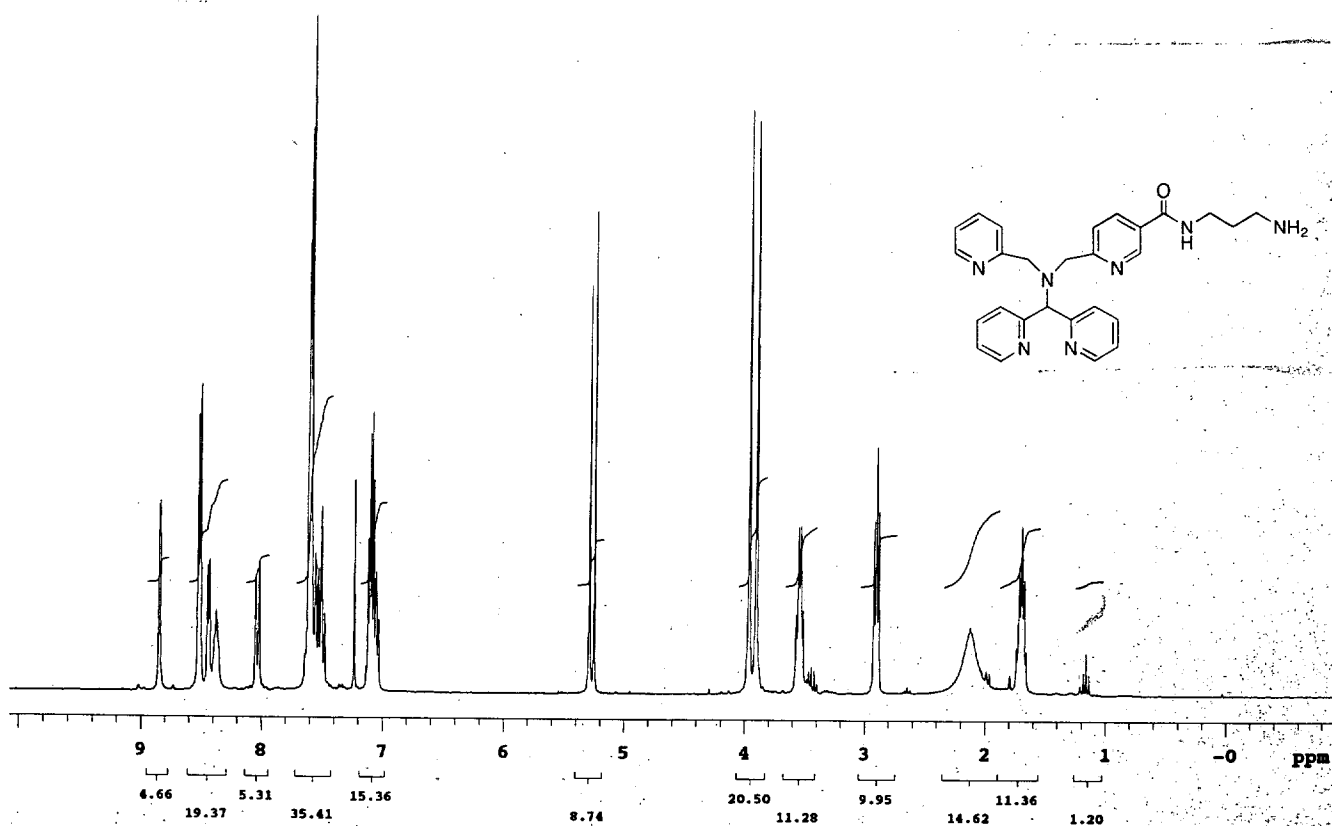
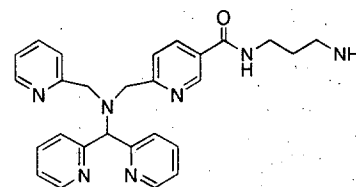




6-(((di-pyridin-2-yl-methyl)-pyridin-2-ylmethyl-amino)-methyl) nicotinic acid methyl ester



*N*-(3-amino-propyl)-6-(((di-pyridin-2-yl-methyl)-pyridin-2-ylmethyl-amino)-methyl) nicotinamide (L2)



12

***N*-(3-(acridin-9-ylamino)-propyl)-6-(((di-pyridin-2-ylmethyl)-pyridin-2-ylmethyl-amino)-methyl) nicotinamide (L1)**

